# DEVELOPMENTS IN HIGH THROUGHPUT ELECTROPHORESIS SYSTEM FOR DNA SEQUENCING AND LARGE FRAGMENT ANALYSIS

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We have been investigating the design alternatives and fabrication technologies necessary for building highly integrated, high throughput DNA sequencing and large fragment analysis systems based on electrophoresis. Our ultimate objective is to develop a DNA analysis system that will sequence up to 3000 clones per 24 hours of operation. Our preliminary design for such an ultimate sequencer uses 384 lanes per system and is capable of resolving greater than 500 bases per lane. We are using microfabrication techniques to build arrays of electrophoresis microchannels on glass substrates. This is an alternative technology[1] to bundles of discrete capillaries that are being investigated by others[2,3,4,5,6]. We believe the microfabrication approach will allow the assembly of a more physically robust system and provide the foundation for ultimately integrating chemical micro-reaction chambers and other fluidic hardware to allow more automated processing of DNA samples for sequencing.

In 1994 we reported the fabrication of 48 channels (1 mm wide, 0.2 mm deep and 25 cm long) etched in large glass plates (25 cm x 42 cm) that resolved about 500 bases per channel[7]. These were used in a 'hybrid' mode where adjacent channels were not physically sealed but separated by a thin layer (~50 μm) of gel. To significantly increase the density of microchannels on a practical size glass plate we have had to develop sealed microchannels to prevent "cross-talk" of DNA among adjacent channels. We have bonded glass plates with etched microchannels which are 10 cm x 10 cm. Realizing that longer channels are desirable to obtain long base reads (e.g. greater than 500 bases), we have also built and are testing the performance of alternative geometries for separation columns (e.g. serpentine loops vs. straight channels). Serpentine or looped channels can be used to arbitrarily extend the overall length of channels at the cost of packing density of channels and some loss of resolution depending upon the number of turns per column[8]. We will discuss experimental results as well as electric field modeling results which have suggested modifications of the geometry of the bends can reduce band spreading at column turns. The trade off of increased channel length necessary for extended base read and the number of channels per substrate has led to a continuing effort to develop a viable bonding process for larger glass plates. In an effort to meet the requirement of extended base read while considering practical limitations on large area bonding we have also developed a model of electrophoretic resolution to help us better understand and optimize the design of our separation channels. We will report on results obtained using this model and discuss the attendant ramifications for the overall system design.

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